

Assessing Stress Responses in Beaked and Sperm Whales in the Bahamas

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LONG-TERM GOALS

This project is developing the use of fecal steroid hormone assays to assess stress responses in Blainville's beaked whales (*Mesoplodon densirostris*, BBW) and sperm whales (*Physeter macrocephalus*) residing in the northern Bahamas. These species were chosen to include a particularly acoustically-sensitive cetacean (beaked whales) and a co-occurring species (sperm whales) for comparison. The goal is to determine baseline fecal hormone levels for reference populations of these two deep-diving whale species, characterizing the natural variations in stress-related hormones according to life history stage (age, sex, reproductive status). The results of this project will set the stage for future research comparing levels of the same stress-related hormones in beaked and sperm whales inhabiting the nearby U.S. Navy Atlantic Undersea Test and Evaluation Center (AUTC) or other habitats with known acoustic exposures from man-made sounds. This approach uses quantifiable alterations in stress-related fecal hormones to determine whether anthropogenic noise is causing measurable physiological changes that can potentially lead to biologically significant effects on individuals and populations.

OBJECTIVES

The objectives of this research project are to:

- (1) Conduct dedicated fecal sampling surveys for populations of beaked and sperm whales off southwest Great Abaco Island and refine methods to maximize sampling rates. Surveys are accompanied by concurrent photo-identification to identify individual whales.

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- (2) Validate radioimmunoassays for fecal metabolites of reproductive hormones (estrogens, progestins, androgens) adrenal GCs and thyroid hormone (T3) for beaked and sperm whales.
- (3) Characterize baseline levels of these five fecal hormones in beaked whales and sperm whales, and describe the natural variation of these hormones according to life-history state (sex, age, reproductive state).

APPROACH

This project is a collaboration between the New England Aquarium (NEAq) and the Bahamas Marine Mammal Research Organization (BMMRO). It includes 1 month of dedicated fecal sample collection in the Bahamas (FY 2011) to prove the feasibility of fecal sampling, and test methods to maximize sample collection rates, from free-ranging beaked and sperm whales off Great Abaco Island in the Bahamas. Fecal sample collection is being conducted by BMMRO scientists (D. Claridge, C. Dunn) with assistance from NEAq scientists (R. Rolland, K. Hunt, S. Kraus). BMMRO is providing individual identification (based on photo-identification) and life history information on sampled whales. The FY 2011 fieldwork was conducted under Bahamian Research Permit (permit no. 12) issued to the BMMRO under authorization of the Bahamas Marine Mammal Protection Act 2005, with approval from the BMMRO and NEAq IACUCs.

In FY 2012, fecal hormone assays will be validated, conducted and interpreted at the Marine Stress Laboratory at the NEAq (R. Rolland, K. Hunt, S. Kraus). A combination of newly collected and archived fecal samples from both species will be used for these studies. Fecal samples will be processed and steroids extracted according to the methods in Rolland et al. (2005). Radio-immunoassays (¹²⁵I and ³-H) for fecal estrogen, progesterone, testosterone, corticosterone and thyroxine (T3) will be validated for beaked and sperm whales using standard parallelism and accuracy studies (Diamandis and Christopoulos 1996). Validation methods for the reproductive and adrenal steroid hormones are detailed in Rolland et al. (2005) and Hunt et al. (2006), and validations for T3 will follow Wasser et al. (2010). Fecal hormone assay results will be used to develop baseline levels for all five hormones in Blainville's beaked whales and sperm whales, including characterization of the natural variation in hormones according to life history state for both whale species using standard descriptive statistics and tests. Additionally, Peter Corkeron (consultant) will undertake statistical analyses of hormone levels from fecal samples in order to assess how these hormones can be used to make inferences regarding stress in beaked and sperm whales. Statistical methods to be used will include machine learning techniques (such as conditional inference trees) and mixed-effects models.

Two Option years include another 30 days of fecal sampling both species in the Bahamas (FY 2013) to increase sample sizes from whales of different life-stages, and a final year (FY 2014) to assay the newly collected samples, complete data analyses and interpretation, and write the final project report.

WORK COMPLETED

Task 1. Fieldwork

During July 2011, fecal samples were collected from free-ranging Blainville's beaked whales and sperm whales off southwest Great Abaco Island using methods previously employed by BMMRO with modifications made by NEAq scientists. Sample collection was accompanied by photo-identification, and photographs were later compared to existing identification catalogues to provide information on

the sampled individual's age and sex class. Beaked whales defecate subsurface in the water column, and one to two divers were towed alongside the research vessel to follow whale movements subsurface (~3-4 m down; Figure 1), and to dive to collect samples when defecation was observed. Modifications to the BMMRO collection method included towing a second diver, and increasing the distance of the divers from the boat hull to increase whale visibility and improve the odds of retrieving samples. Several sample collection devices were compared including a turkey baster, a Slurp gun (used to collect tropical fish), a small fish collection net and/or a Ziplock bag. Sperm whales defecate at the surface, and samples were scooped from the water surface using a 30cm diameter hoop and handle with a 300- micron nylon mesh net (Sea-Gear, Inc.) attached to a pole or by simply collecting directly in one or more plastic storage containers. Samples were placed in plastic jars, placed in a cooler on ice-packs, and frozen at -20 °C after returning to the field station.



Figure 1. Beaked whale samples are collected sub-surface by divers towed alongside the research vessel.

Dedicated sampling surveys were conducted during 27 vessel-days using 5.7 and 6.8m rigid-hulled inflatable boats. Surveys were primarily concentrated near the 1000 m isobaths along the southwest side of Great Abaco Island, in the northern Bahamas (Figure 2). Survey effort totaled over 2000 km of vessel track lines.

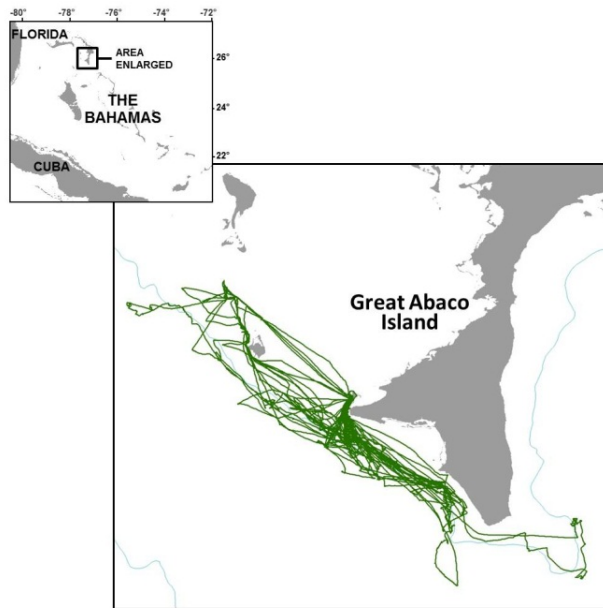


Figure 2. Map showing vessel tracks (green lines) during surveys off the coast of Great Abaco Island during July 2011. Survey efforts were concentrated off the southwestern side of the island along the 1000 m isobaths (shown as blue line).

Archived Samples

Previously collected archived (-80 °C) frozen fecal samples from Blainville’s beaked whales and sperm whales housed at BMMRO and at Woods Hole Oceanographic Institution were examined for appropriate preservation methods (i.e., no preservative) for fecal hormone assays, and individual whale life history data were obtained when available.

RESULTS

During surveys, there were 60 cetacean sightings, including six different species. The research team located 13 groups of Blainville’s beaked whales and 7 groups of sperm whales, and spent a total of 22.0 hours and 15.5 hours, respectively, following groups. Group size ranged from 2-7 whales for Blainville’s beaked whales (median = 4) and 1-14 animals for sperm whales (median = 5).

Fecal samples were collected from throughout the survey area (Figure 3), with the majority from the southwest side of Great Abaco Island where survey effort was concentrated.

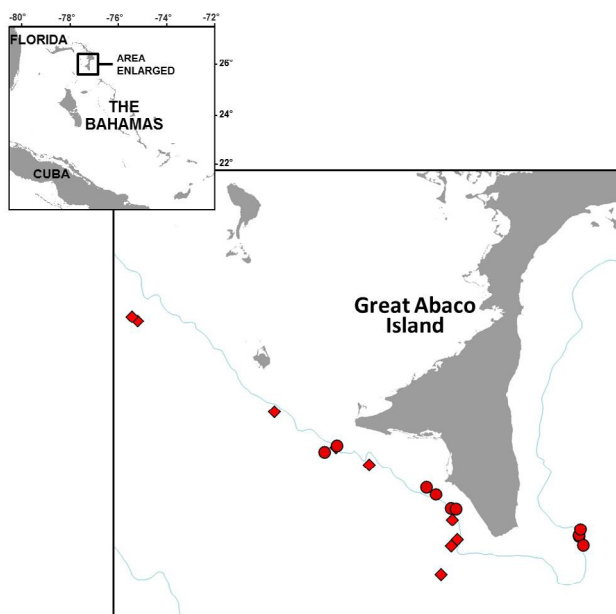


Figure 3. Map showing that fecal samples were collected from throughout the area surveyed off Great Abaco Island during July 2011. Blainville's beaked whale sample locations are represented by red circles and sperm whales by red diamonds. The 1000 m line is shown.

Nineteen fecal samples were successfully collected from Blainville's beaked whales ($n = 10$) and sperm whales ($n = 9$) during the field effort (Table 1). Repeated collections were made from the same individuals on 3 occasions, one of which was 4 days after the first collection was made. Up to 5 samples were collected in a day of survey. The success of beaked whale sampling, in particular, is dependent upon a Beaufort seastate ≤ 2 and good underwater visibility.

Table 1. Summary of collection data for fecal samples.

SAMPLE COLLECTION DATA						
Sample #	Date	Enc. Seq.	Collection Time	Latitude (N)	Longitude (W)	Whale ID
<i>Blainville's beaked whales</i>						
Md 11-001	7/6/2011	3	1450	2557.687	7726.845	New
Md 11-002	7/10/2011	4	1627	2558.206	7725.813	New
Md 11-003	7/11/2011	1	1804	2553.066	7716.436	New
Md 11-004	7/13/2011	1	1238	2554.825	7718.464	see Notes
Md 11-005	7/15/2011	1	1427	2553.003	7716.017	Md135
Md 11-006	7/16/2011	1	857	2554.217	7717.665	Md135
Md 11-007	7/16/2011	5	1411	2550.733	7705.915	Md091

Table 1. Summary of collection data for fecal samples (continued).

Md 11-008	7/16/2011	5	1427	2550.855	7705.915	Unknown
Md 11-009	7/16/2011	6	1539	2551.306	7705.793	Md139
Md 11-010	7/16/2011	6	1618	2550.017	7705.553	Md139
<i>Sperm whales</i>						
Pm 11-001	7/19/2011	2	1620	2536.693	7723.193	Unknown
Pm 11-002	7/20/2011	2	1350	2601.090	7730.976	Unknown
Pm 11-003	7/20/2011	2	1550	2558.109	7725.893	Unknown
Pm 11-004	7/21/2011	1	845	2552.145	7716.345	Pm150?
Pm 11-005	7/21/2011	1	943	2550.507	7715.928	Pm080?
Pm 11-006	7/21/2011	1	955	2549.996	7716.421	Unknown
Pm 11-007	7/21/2011	1	1128	2547.607	7717.265	New
Pm 11-008	7/26/2011	2	1320	2608.583	7742.223	Pm024?
Pm 11-009	7/26/2011	2	1340	2608.944	7746.699	Unknown

Four additional samples were seen but we were unable to collect them for a variety of reasons. All of the beaked whale samples were collected within 4 m of the surface; only one sample was too deep (>10 m) for the diver to retrieve it. We tried several collection methods for the beaked whale feces and discovered that using a combination of a large Ziplock bag and a small hand-held dipping net worked best. We did not have much success using the 300-micron mesh net for collection of sperm whale feces because the mesh size was not small enough. The most successful method was to locate a clumped portion of the sample floating at the surface and scoop the feces directly into the plastic sampling container.

Collection data including information on the age and sex class and reproductive status based on individual life histories for known whales are summarized in Table 2. Samples were collected from all age classes, including several lactating females. Additional life history information will be available for the sperm whale samples in FY12 as images are matched to the catalog. Two samples were collected (same day) from an adult female Blainville's beaked whale that had been tagged with a LIMPET dart-tag on June 5th, 2011. The fecal collection took place on July 16th, at which time the tag was still transmitting but had almost fallen out.

Table 2. Summary of individual life history data for known whales.

INDIVIDUAL LIFE HISTORY DATA					
Sample #	Whale ID	Sex*	Age class	Reproductive State	Notes
<i>Blainville's beaked whales</i>					
Md 11-001	New	F	Subadult	Immature	

Table 2. Summary of individual life history data for known whales (continued).

Md 11-002	New	F	Subadult	Immature	Same as Md 11-001
Md 11-003	New	M	Adult	Mature	
Md 11-004	see notes	F	Adult	Lact. F or Mature	Either Md135 or Md121
Md 11-005	Md135	F	Adult	Lactating F	With neonate
Md 11-006	Md135	F	Adult	Lactating F	Same as Md 11-005
Md 11-007	Md091	F	Adult	Lactating F	With calf 2-3 yrs old
Md 11-008	New	M	Subadult	Immature	
Md 11-009	Md139	F	Adult	Lactating F	With calf 2 yrs old. Limpet tag.
Md 11-010	Md139	F	Adult	Lactating F	Same as Md 11-009. Limpet tag.
<i>Sperm whales</i>					
Pm 11-001	Unk	Unk	Subadult	Immature	
Pm 11-002	Unk	Unk	Juvenile	Immature	
Pm 11-003	Unk	F	Adult	Mature	
Pm 11-004	Pm150?	Unk	Subadult?	Immature?	
Pm 11-005	Pm080?	Unk	Subadult	Immature	First seen in 2000
Pm 11-006	Unk	Unk	Subad/Juv.	Immature	Small whale
Pm 11-007	New	Unk	Juvenile	Immature	
Pm 11-008	Pm024?	Unk	Subadult?	Immature?	First seen in 1997
Pm 11-009	Unk	Unk	Subadult?	Immature?	

Archived samples

An additional 21 fecal samples archived at WHOI and BMMRO were properly stored and will be used for hormone assays. This includes 4 beaked whale samples (collected in 2001, 2004, 2010), and 17 sperm whale samples (collected in 2001, 2003, 2004, 2010). Life history data on the sampled whales are available for many of these samples. Including both the newly collected and archived fecal samples, the total samples available for hormone analyses in FY 12 is 14 beaked whales samples and 26 sperm whale samples.

IMPACT/APPLICATIONS

The first phase of this project successfully demonstrated the feasibility of collecting fecal samples from free-ranging beaked and sperm whales in the Bahamas to use for fecal hormone analyses. Samples were collected from a variety of catalogued individual whales of both sexes and in different reproductive states. Repeat collections from individual whales was also accomplished. Therefore, the collectability of fecal samples makes it a viable matrix to validate assays for stress-related hormones

with the advantage of that samples are obtained non-invasively causing no disturbance to the research subjects.

RELATED PROJECTS

No related projects.

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